ASSESSMENT OF MICROBIAL LOAD OF RAW MEAT AT ABATTOIRS AND RETAIL OUTLETS

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ABSTRACT

Aim of the present study was to assess the microbial load of raw meat at abattoirs and retail outlets in different areas of Lahore. Beef, mutton (sheep, goat) and chicken meat samples (n=140) were collected from various abattoirs (n=60) and retail outlets (n=80). All the samples were subjected to aerobic plate count (APC), *E. coli* count, *Staphylococcus aureus* count and *Salmonella* detection. Mean APCs of beef, sheep, and goat meat from abattoirs (5.35, 5.42 and 4.84 log10 CFU/cm^2 respectively) were significantly lower as compared to APC values of retail outlets (7.15, 6.92 and 6.62 log10 CFU/cm^2 respectively). Mean APC of chicken meat from retail outlets was 7.22 log10 CFU/cm^2. Mean *E. coli* counts for the beef, sheep and goat meat from abattoirs and retail outlet were 2.81, 2.94; 2.64, 2,78 and 2.86, 1.94 log10 CFU/cm^2 respectively, while mean *S. aureus* counts were 2.76, 2.91; 2.90, 2.96 and 2.80, 3.07 log10 CFU/cm^2 respectively. Mean *E. coli* and *S. aureus* counts for chicken outlets were 2.74 and 3.80 log10 CFU/cm^2, respectively. There were no significant differences (p 0.05) between the *E. coli* and *S. aureus* number for the abattoirs and retail outs of beef, sheep and goat meat. The *E. coli*, *S. aureus* and *Salmonella* were detected from total of 45%, 72% and 26% samples respectively. It is concluded that microbial load of raw meat from abattoirs and retail shops in Lahore is high which insinuates its possible role in spoilage and food-borne illnesses.

Key words: Meat, Abattoir, Retail outlets, *E. coli*, *Staphylococcus aureus* and *Salmonella*.

INTRODUCTION

Meat, an excellent source of protein in human diet is highly susceptible to microbial contaminations, which can cause its spoilage and food borne infections in human, resulting in economic and health losses (Komba *et al.*, 2012). Although muscles of healthy animals do not contain microorganisms, meat tissues get contamination during the various stages of slaughter and transportation (Ercolini *et al.*, 2006). A great diversity of microbes inhabit fresh meat generally, but different types may become dominant depending on pH, composition, textures, storage temperature, and transportation means of raw meat (Ercolini *et al.*, 2006; Li *et al.*, 2006; Adu-Gyamfi *et al.*, 2012).

Meat, a rich source of the protein and fat, low in carbohydrate content and with sufficient water activity, supports the growth of both spoilage and pathogenic bacteria. Major spoilage organisms in raw meat and poultry are *Pseudomonas spp.* other may include *Shewanella*, *Brochothrix* and members of enterobacteriaceae. Growth of yeasts and molds is essentially slow on fresh meat as compared to bacteria, therefore, they are not major component of spoilage flora (Doyle, 2007). The food and Agricultural organization (FAO) of the United Nations and the World Health Organization (WHO) state that illness due to contaminated food is perhaps the most widespread health problem and an important cause of reduced economic productivity (Käferstein, 2003). Raw meat may harbour many important pathogenic microbes i.e. *Salmonella spp.*, *Campylobacter jejuni/coli*, *Yersinia enterocolitica*, *E. coli*, *S. aureus* and, to some extent, *Listeria monocytogenes*, making the meat a risk for human health, as without the proper handling and control of these pathogens, food borne ill-nesses may occur (Nørrung *et al.*, 2009).

The meat, available at retail outlets comes through a long chain of slaughtering, and transportation, where each step may pose a risk of microbial contamination. The sanitary conditions of abattoirs and its surrounding environment are major factors contributing in bacterial contamination of meat (Gill *et al.*, 2000). Contaminations can be compounded during transportation, storage and handling of meat at butcher shops.

To control the food-borne illnesses and to keep the microbial load of raw meat in check, the food safety requirements should be followed strictly in accordance with HACCP (Hazard analysis critical control point), but in developing countries like Pakistan, the abattoir environment, its sanitary level, and transportation and storage conditions not only contaminate but also enhance the growth of different types of spoilage as well as pathogenic bacteria in meat.

Present study was designed to assess the microbial load such as *S. aureus* and *E. coli* and *Salmonella* from raw meat at abattoirs and retail outlets in different areas of the Lahore city.

MATERIALS AND METHODS

Sample collection and processing: Beef, sheep, goat and chicken meat samples (n=140) were collected from various abattoirs (n=60) and retail outlets (n=80) in Lahore City. Sampling was carried out by swabbing the muscular surface of fore and hind quarter of each carcass after flying and washing. An area of $100\ cm^2$ marked with a sterile frame of $10\ cm \times 10\ cm$ on each site of the carcass was rubbed for 30 seconds and swabs were transferred to a screw-capped test tube containing 10 ml of sterile maintenance medium (0.85% NaCl and 0.1% peptone) (Bell, 1997). The tubes were transported to lab at 4ºC and processed for further analysis within four hours.

Aerobic plate count: Aerobic plate count was carried out on total plate count agar as described by (Bell, 1997). The medium was autoclaved and maintained at 46ºC. Samples were serially diluted and an aliquot of 1 ml of each of serial dilution was transferred to the petri dishes (4 inch diameter) and molten agar (15-20 ml) was poured on it. Plates were gently swirled to uniformly mix the sample and incubated at 37ºC for 48 hours. After incubation APC was determined from appropriate plates.

Enumeration of *Staphylococcus aureus:* Baird Parker agar (Oxoid, England), a selective medium for the isolation and counting of coagulase positive staphylococci was used for the enumeration of *Staphylococcus aureus* as described by (Bhandare *et al.*, 2007). Enumeration of *S. aureus* was done by spreading an appropriate dilution of sample on agar plates followed by aerobic incubation at 37°C for 48hrs. Further confirmation of *S. aureus* was carried out by grams staining and catalase testing.

Enumeration of *Escherichia coli:* *Escherichia coli* were enumerated on Eosin methylene blue agar (Oxoid, England) by plating an appropriate dilution on plates followed by aerobic incubation at 37°C for 24hrs. After incubation *E. coli* were counted as colonies with distinct metallic sheen (Bhandare *et al.*, 2007).

Isolation and identification of *Salmonella:* Presence of *Salmonella* in meat sample was established by pre-enrichment of meat sample in lactose broth followed by enrichment in tetra-thionate broth and final detection on Bismuth sulphite agar as recommended by WHO procdures.

Statistical Analysis: Microbial counts (CFU/cm^2) were represented as $\log10\ CFU/cm^2$ and means were calculated. Microbial counts were compared by ANOVA using SPSS Software 13.0.

RESULTS AND DISCUSSION

Aerobic Plate counts: Aerobic plate count (APC) is a measure of microbial quality of the meat. Presence of microbes in high numbers (APC $>10^7 CFU/cm^2$) fast tracks the spoilage of the meat. According to the Raw Meat Grading and Marketing Rules (1991, APC of 60% of analyzed samples must not exceed $10^6\ CFU/g$ or cm^2, whereas 40% of the samples may have counts up to 10^7 CFU/g or cm^2 (Mukhopadhyay *et al.*, 2009). Results of mean APCs of beef, sheep, goat and Chicken are presented in table (1). Mean APCs of beef, sheep, and goat meat from abattoirs (5.35, 5.42 and 4.84 log10 CFU/cm^2, respectively) were significantly lower as compared to APC values of retail outlets (7.15, 6.92 and 6.62 log10 CFU/cm^2, respectively). APC of retail chicken samples was 7.22 log10 CFU/cm^2. Mean APCs of beef, sheep and goat meat from abattoirs were not statistically significant ($P < 0.05$), similarly APC level of beef, sheep, goat and chicken meat from retail outlets did not differ significantly ($P < 0.05$). Significantly higher mean APCs for the retail outlets as compared to the abattoirs, indicate the inferior quality of transportation and storage conditions, and supportive environment of retail outlets for the microbial growth.

In present study, 51% of samples had APC more than 6 log10 CFU/cm^2, which indicates highly contaminated meat. The condition was acceptable, only for the sheep and goat abattoirs, which had 40% and 30% of samples with APC more than log10 6.00 CFU/cm^2 respectively. Higher level of aerobic plate count in this study is in accordance with previous studies (Alvarez-Astorga *et al.*, 2002; Bhandare *et al.*, 2007; Haque *et al.*, 2008; Hassan *et al.*, 2010). Significantly higher level of contamination in the meat shops as compared to the abattoir have also been reported previously (Bhandare *et al.*, 2007). Although the microbial contamination of abattoirs was lower as compared to the retail outs, it was higher as compared to reports from developed countries and do not conform to EU specifications (Gill *et al.*, 2000; Duffy *et al.*, 2001).

***E. coli* count:** E. coli count in raw meat presented in table (2) indicates the hygiene qualities of meat. In this study, we only detected and enumerated the *E. coli* irrespective of pathogenic or nonpathogenic strain to estimate the level of hygiene. Out of 140 samples, *E. coli* were present in total of 63 (45%) samples including abattoirs (18) and retail outlets (45) which were higher than established limits in guidelines (Alvarez-Astorga *et al.*, 2002). Similar results have also been reported for retail chicken (>90% incidence of *E. coli*) in Australia (Pointon *et al.*, 2008). Mean *E. coli* counts for the beef,

sheep and goat meat from abattoirs and retail outlet were 2.81, 2.94; 2.64, 2.78 and 2.86, 1.94 log10 CFU/cm^2 respectively. *E. coli* positive samples were significantly higher for beef outlets as compared to beef abattoirs (75% vs 40%), sheep outlets as compared to sheep abattoirs (55% vs 30%), and goat outlets as compared to goat abattoirs (50% vs 20%). The 45% of the chicken samples collected from retail outlets were also positive for *E .coli* with mean *E. coli* counts of 2.74 log10 CFU/cm^2. The presence of *E. coli* strains in meat and meat products have been studied by many researchers (Dutta *et al.*, 2000; Alvarez-Astorga *et al.*, 2002; Bhandare *et al.*, 2007; Doyle, 2007; EFSA, 2007; Adu-Gyamfi *et al.*, 2012).

***Staphylococcus aureus* count:** Staphylococci, which are natural flora of skin and mucous membranes of animals and human can cause meat contamination (Nørrung *et al.*, 2009). In present study, mean *S. aureus* counts for the beef, sheep and goat meat from abattoirs and retail outlet were 2.76, 2.91; 2.90, 2.96 and 2.80, 3.07 log10 CFU/cm^2 respectively (Table 2). *S. aureus* was isolated from the 72 (51%) samples, which indicate poor sanitary quality of abattoirs and retail outlets. *S. aureus* positive samples were significantly higher for beef outlets as compared to beef abattoirs (70% vs 55%), sheep outlets as compared to sheep abattoirs (45% vs 25%), and goat outlets as compared to goat abattoirs (70% vs 40%). The 55% of

the chicken samples collected from retail outlets were also positive for *S. aureus* with mean *S. aureus* counts of 3.08 log CFU/cm^2. Significantly higher percentage of sheep and goat samples from retail outlets were positive for the *S. aureus* as compared to samples from abattoirs. The results of the present study are in agreement with the previous findings (Haque *et al.*, 2008; Tassew *et al.*, 2010). Higher level of microbial contaminations including *S. aureus* of meat has also been reported previously (Voidarou *et al.*, 2011).

Salmonellae detection: Out of 140 samples, Salmonellae were detected from 26 samples including abattoirs (08) and retail outlets (18). Salmonellae positive samples were not significantly different for beef outlets as compared to beef abattoirs (35% vs 20%), sheep outlets as compared to sheep abattoirs (10% vs 10%), and goat outlets as compared to goat abattoirs (10% vs 10 %) respectively. The 25% of the chicken samples collected from retail outlets were also positive for Salmonellae. The high prevalence of *Salmonella* can be attributed to the contaminated waters used in abattoirs for carcass washing. Salmonella has frequently been isolated from the abattoir environments and gastrointestinal tract of all farmed and wild animals, especially poultry (EFSA, 2007; Nørrung *et al.*, 2009). Pointon *et al* (2008) have also reported high incidence of *Salmonella* from retail chicken in two Australian states (47.7 and 35.5%).

Table 1 Aerobic Plate Counts of different meat types as represented by log10 CFU/cm^2

log CFU/cm^2	Beef		Sheep		Goat		Chicken	Total
	Abattoir (n= 20)	R. Outlets (n= 20)	Abattoir (n= 20)	R. Outlets (n= 20)	Abattoir (n= 20)	R. Outlets (n= 20)	R. Outlets (n= 20)	(n= 140)
	n[a](%)	n[a](%)	n[a](%)	n[a](%)	n[a](%)	n[a](%)	n[a](%)	
>4 to <5	4 (20)	1 (5)	7 (35)	1 (5)	6 (30)	1 (5)	5 (25)	25 (18)
>5 to <6	6 (30)	5 (25)	5 (25)	7 (35)	8 (40)	7 (35)	6 (30)	44 (31)
>6	10 (50)	14 (70)	8 (40)	12 (60)	6 (30)	12 (60)	9 (45)	71 (51)
Mean± S.D	5.35 1.15	7.15 ± 2.45[*]	5.42±1.34	6.92 ± 2.16[*]	4.84±1.17	6.62 ± 1.12[*]	7.22 ± 2.11	5.50±3.12

[a] number of samples with CFU/cm^2 corresponding to the first column of same row
[*] significantly higher values for retail outlets as compared to abattoirs for a group ($P < 0.05$)

Table 2 *Colifoms*, *Staphylococcus aureus* and *Salmonella* profile of meat as represented by number of positive samples and mean log CFU/cm^2

Type Of Meat	Sampling type	No of samples	E. coli		S. aureus		Salmonella
			n[a] (%)	(Mean ±log CFU/cm^2)	n (%)	(Mean± log CFU/cm^2)	n (%)
Beef	Abattoir	20	8 (40)	2.81±1.04	11 (55)	2.76±1.30	4(20)
	R. Outlets	20	15 (75)[*]	2.94±1.45	14(70)	2.91±1.28	7(35)[*]
Sheep	Abattoir	20	6 (30)	2.64±1.66	5(25)	2.90 ±1.55	2(10)
	R. Outlets	20	11 (55)[*]	2.78±1.10	9(45)[*]	2.96 ±1.66	2(10)
Goat	Abattoir	20	4 (20)	2.86±0.86	8(40)	2.80 ±1.53	2(10)
	R. Outlets	20	10 (50)[*]	1.94±1.12	14 (70)[*]	3.07 ±1.45	2(10)
Chicken	R. Outlets	20	9 (45)	2.74±1.13	11(55)	3.80 ±1.34	7(25)
	Total	140	63 (45)		72 (51)		26 (19)

[*]Significantly different when compared with the above row in same column at ($P < 0.05$)
[a] Number of positive samples for specific organism in each group

It is concluded that microbial load of raw meat in Lahore is high which can be attributed to unhygienic conditions in slaughter houses and transportation. It is suggested that authorities should closely monitor and regulate proper slaughtering and transportation facilities in Lahore.

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